

# Bulletin of the Agricultural Chemical Society of Japan.

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# ON COLOUR REACTIONS OF DIBASIC ACIDS OF FATTY SERIES, ESPECIALLY OF FUMARIC ACID.

By Teizo TAKAHASHI and Kinichiro SAKAGUCHI.

(Received June 28th., 1926.)

On the colour reaction of fumaric acid as far as we know just one<sup>(1)</sup> is acquainted with to give a red colouration when the acid is reacted with diazo-benzenesulphonic acid. However the delicacy of it is not sufficient enough to be relied upon as a sole reaction. The colour reactions described below offer a more satisfactory means of identification.

## FIRST REACTION.

As the reagents required are resorcinol and sulphuric acid, the procedure is already known in the case of succinic acid.<sup>(2)</sup>

A piece of fumaric acid of the size of a pin's head is placed in a test tube with about ten times its quantity of powdered resorcinol and 1-1.5 c.c. of concentrated sulphuric acid then heated gently in the beginning and later briskly until it attains nearly 190-195°C. Whereby the liquid becomes red with a green fluorescence. After cooling add some water and heat again.

Cool well and a few drops of the reaction products are introduced drop by drop into ammonia, which colours red with a green fluorescence.

This reaction is to be given by the dilute solution of fumaric acid, thus:—A few drops of a 0.1 % solution of fumaric acid placed in a test tube with equal drops of a 0.1 per cent solution of resorcinol and 1-1.5 c.c. of concentrated sulphuric acid gives a liquid which on treating as mentioned above shows a yellow colour with green fluorescence.

Anhydride: i. e. maleinic anhydride gives quite the same colouration as met with by crystals of fumaric acid.

Succinic acid gives a yellow colouration in the first part of the reaction and when introduced into ammonia it changes to a green fluorescent liquid.

Malic acid on treating as above gives a liquid which on heating becomes at first yellow and then crimson-red even after the addition of water. A blueish violet colour is produced by adding 2 or 3 drops of this crimson-red colour fluid into ammonia.

This colouration is quite specific to malic acid and may distinguish this

(1) Hans Einbeck: Zeit. f. Phys. Chem. Bd. 90, S. 306, 1914.

(2) L. Rosenthaler: Nachweis Org. Verb. S. 331.

from succinic- and fumaric acids.

Tartaric acid. Mohler's reaction<sup>(3)</sup> for the detection of the acid is analogous to ours in applying the same reagents, although we applied a higher temperature than in his case.<sup>(4)</sup> No fluorescence is observed when the reaction product is added to ammonia.

Citric acid. Our reaction is a new one to this acid. On treating the crystals of citric acid of the size of a pea as in the case of tartaric acid a liquid is obtained which on heating becomes at first pink-red and then yellow by a prolonged heating. Pink-red colour recovers when it is diluted with water. Finally when a few drops of the liquid is added to ammonia the latter takes a blueish-green fluorescence with violet shade, this is specific to the citric acid.

Beside these dibasic acids gluconic-, mucic-, saccharic-, and lactic acids behave in a very similar way. These reactions will be described in the other paper.

#### SECOND REACTION.<sup>(5)</sup>

This is based on the colour produced when the acid to be tested is reacted with  $\alpha$ -naphtol in the presence of concentrated sulphuric acid.

The procedure is quite same as it is mentioned in the first reaction, where resorcinol is used.

Fumaric acid. This acid gives a green colour when the reagents are just mixed. By gently heating it becomes at first yellow then pink-red and finally brown-red which changes to an opaque brown liquid after a long standing. Red colour is restored by the addition of water to the liquid. When this liquid, after shaking is introduced drop by drop into ammonia a yellow-green colouration is perceived. After standing the reddish shade intensifies with fluorescence.

The change of reaction given by the other acids will be shown in the table below :-

| Acid.   | When they are mixed. | By heating.  | Colour produced by ammonia.              |
|---|----------------------|--|--|
| Succinic acid.  | Green.               | Dark and light red.  | Light greenish-yellow with fluorescence. |
| Malic acid.   | —                    | Crimson-red and by addition of water it alters to opaque fluid.        | Red with fluorescence.                   |
| Tartaric acid<br>(Pinerūa's reaction :-<br>First blue then green) | Green.               | Yellow $\rightarrow$ light pink red yellow $\rightarrow$ greenish blue | Light yellow.                            |
| Citric acid.  | —                    | Green $\rightarrow$ crimson-red.                                       | Pink red.                                |

## THIRD REACTION.

In this case  $\beta$ -naphtol is used instead of  $\alpha$ -naphtol employed in the second reaction. The first part of this reaction is already demonstrated by Pinërūa<sup>(6)</sup> to detect citric acid, which gives a blue color according to his description. For citric acid however the delicacy of the reaction is not sufficient enough as we have experienced with fumaric acid.

A small piece of fumaric acid of the size of pin's head is put in a test tube with  $\beta$ -naphtol and sulphuric acid.

The yellowish-green mixture on heating first colorless then dark-red and finally turns to a dark-green opaque liquid. After cooling and diluting with water, the red color goes into the aqueous layer leaving a green layer underneath it. On shaking the green color is restored with fluorescence which increases when the liquid is added to ammonia.

The colourations given by the other acids by this reagent are tabulated below :-

| Acid.          | When they are mixed.                 | On heating.  | By adding water.      | Colour given by ammonia.           |
|----------------|--------------------------------------|--|-----------------------|------------------------------------|
| Succinic acid. | Yellowish-light green colouration    | Becomes colourless then dark violet and finally greenish violet (Less delicate than fumaric acid). | Water colours yellow. | Green fluorescent fluid.           |
| Malic acid.    | Colourless or light greenish yellow. | From light yellowish to red. A fluorescence while yellow. (Distinction from lactate).              | Red.                  | Light yellow with fluorescence.    |
| Tartaric acid. | Colourless.                          | First green then to dark bluish-green.   | Darkness increases.   | Coloured yellow with fluorescence. |
| Citric acid.   | —                                    | Light dark blue. Green after cooling. (Blue after Pinërūa).  | Becomes yellowish.    | Light green with fluorescence.     |

## FOURTH REACTION.

This reaction is given by hydroquinone and sulphuric acid. The reaction is quite same as in the foregoing reactions. Fumaric acid gives no color

(3) Mohler's reaction : Bull. Soc. chim. France [3] 4 (1890), 728.

(4) Mohler heated to 130°C, but in our case it should be heated to 190-195°C.

(5) Pinërūa's (1897) Colour reaction of tartaric acid which becomes first blue then green when reacted with  $\alpha$ -naphtol and sulphuric acid.

just when the reagents are mixed. On heating gently at first a light-red then a dark-red color appears. By the addition of water it changes to yellow. The end color reaction given when the product is added to ammonia is yellow. This reaction is especially preferable to identify succinic acid from fumaric acid which accompanies the former as an impurity.

Following table shows a distinction of colors given by the other acids:-

| Acid.          | When they are mixed. | On heating.  | By adding water.   | Colour given by ammonia.                                     |
|----------------|----------------------|--|--------------------|--|
| Succinic acid. | Colourless.          | First yellow then dark green (Distinction from fumaric acid.)                    | Dark-yellow.       | Fluorescent yellowish-green (Distinction from fumaric acid.) |
| Malic acid.    | Colourless.          | First yellow then blood red.   | Redness increases. | Dirty yellow.  |
| Tartaric acid. | Colourless.          | First yellow with evolution of gas, then reddish-yellow and finally crimson red. | —                  | Orange yellow.   |
| Citric acid.   | Colourless.          | Yellow at first then dark green.   | —                  | Yellowish-green with fluorescence.                           |

#### SUMMARY.

1) The first reaction given by dibasic acid of fatty series in the presence of resorcinol and concentrated sulphuric acid, is a quite new one as to the fumaric acid.

Fumaric acid gives red-color with green fluorescence and by this color is distinguishable from succinic acid which gives just a green fluorescence by the same treatment.

The colourations given by malic, tartaric and citric acids distinguish them from each other most conveniently.

2) The color reaction given by  $\alpha$ -naphtol and concentrated sulphuric acid (second reaction) is a new one for fumaric acid (green to red). Succinic acid shows less delicacy than fumaric acid. So it is most conveniently advisable to use this reaction for the identification of either fumaric or succinic acid, especially when the former is present in succinic acid as an impurity. Tartaric acid gives no fluorescence in the last colouration in this reaction as experienced in the first reaction. Citric acid may be distinguished from other acid only by the absence of fluorescence in the last colour.

3) Green colour with fluorescence given by fumaric acid in presence of  $\beta$ -naphtol and sulphuric acid is a new reaction as far as related to this acid. As to succinic, malic, tartaric and citric acids the colouration given by the same procedure is quite specific to each of these acids. The distinction of

succinic acid from fumaric exists in the purplish tinge by the former in the stage of heating in the procedure. Both malic and tartaric acids distinguish from fumaric, succinic and citric acids by the fluorescent light yellow colouration in the end of the reaction. Malic acid characterizes itself from tartaric acid by a yellowish-red or a fluorescent yellow colour in the stage of heating and a red colour by the addition of water in the procedure i. e. the colouration due to the latter is dark bluish-green on heating and dark when diluted with water.

4) The fourth colour reaction given by fumaric acid in presence of hydroquinone and sulphuric acid is a new one with regard to this acid. By the same procedure succinic acid gives a fluorescent yellowish-green colouration which is easily recognisable even accompanied with yellow colour to be produced by fumaric acid if present as an impurity. Malic, tartaric and citric acids show a very delicate and characteristic colouration by this procedure.

(This is reported already in "Journal of the Agric. Chem. Society of Japan." Vol.

No. 14, 1925.1)

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## ON THE PRESENCE OF THE URIC ACID IN THE PUPA OF TUSSAH SILKWORM (*ANTHEREA PERNYI*).

By Jiro KATO.

(From the Central Laboratory, S. M. R. Co. Dairen, South Manchuria.)

(Received Aug. 9th., 1926.)

There are many researches on the chemical constitution of silkworm at different stages of its metamorphosis, but no research on tussah silkworm. So we tried to study the chemical constitution of the pupa of tussah silkworm, and learned that the pupa contains a tolerably large quantity of uric acid. We determined its uric acid content by estimating the soluble matter, soluble nitrogen, and uric acid from nitrogen at the different stages of pupa, and the uric acid content was found to be about 1 % at the beginning and 4 % at the end.

As tussah silkworm passes a winter in the pupa stage, this stage durates about 200 days and the metabolism is carried on without taking food. Therefore, one can imagine that there is increase of the uric acid content in the pupa body as experienced in this work. It is very interesting as a new

utilizing method of the pupa, if the uric acid is prepared in relatively good yield such as 4% or so.

#### EXPERIMENTAL PART.

##### 1. Sampling.

We bought tussah silk cocoon at Antung, Oct., 1925. At the intervals of 30 days, some pupa were taken out from the cocoons & only those with healthy appearance were weighed, dried powdered & put to the analysis. The date in which the sample was taken is as follows :-

| No.                                     | 1                                 | 2    | 3    | 4     | 5    | 6    | 7        |
|---|-----------------------------------|------|------|-------|------|------|----------|
| No. of days                             |                                   |      |      |       |      |      |          |
| Date.                                   | Oct.                              | Dec. | Nov. | Jan.  | Feb. | Mar. | Apr.     |
|   | 25.                               | 24.  | 24.  | 23.   | 22.  | 24.  | 10-20.   |
|   | 1925.                             |      |      | 1926. |      |      |          |
| No. of days after pupation. (rough no.) |                                   |      |      |       |      |      |          |
|   | 30.                               | 60.  | 90.  | 120.  | 150  | 180. | 200-200. |
| (Remarks :-                             | 1-6. Pupa powder. 7. Moth powder. |      |      |       |      |      |          |

##### 2. Total nitrogen, soluble and insoluble nitrogen.

The total nitrogen was determined by Kjeldahl's method. To estimate the soluble nitrogen, 2 gms. of the sample were weighed with hot water, repeatedly and filled up to 2 liters, and a definite quantity of this extract was taken and its nitrogen was determined. The difference of these two nitrogens is calculated as to be the insoluble nitrogen. This experiment gave the following results :-

|                      | 1     | 2     | 3     | 4     | 5     | 6     | 7      |
|----------------------|-------|-------|-------|-------|-------|-------|--------|
| % in fresh matter.   |       |       |       |       |       |       |        |
| Total N.             | 2.24  | 2.45  | 2.52  | 2.48  | 2.52  | 2.62  | 3.32   |
| Soluble N.           | 0.80  | 0.74  | 0.91  | 1.02  | 1.08  | 1.11  | 0.58   |
| % in dry matter      |       |       |       |       |       |       |        |
| Insol. N.            | 1.64  | 1.72  | 1.61  | 1.46  | 1.44  | 1.51  | 2.74   |
| Total. N.            | 10.07 | 10.75 | 11.37 | 11.05 | 11.34 | 12.21 | 11.005 |
| Sol. N.              | 3.31  | 3.23  | 4.12  | 4.56  | 4.83  | 5.18  | 1.93   |
| Insol. N.            | 6.76  | 7.52  | 7.25  | 6.49  | 6.51  | 7.03  | 9.12   |
| % in total nitrogen. |       |       |       |       |       |       |        |
| Sol. N.              | 33.03 | 30.05 | 36.24 | 41.27 | 42.59 | 42.42 | 17.499 |
| Insol. N.            | 66.97 | 69.95 | 63.76 | 58.73 | 57.41 | 57.41 | 82.53  |

From the above results, it is distinct that the soluble nitrogen increases according as the course of the pupa stage. The increase of soluble nitrogen may be due to the increase of the substances of lower molecular weight such as the amino acids, bases, soluble proteins, uric acid, & produced by the decomposition of the insoluble nitrogenous matter. But this increase is chiefly due to the uric acid, as it can be proved by other experiment that

the amino acids, bases and soluble proteins are not increased markedly.

### 3. Soluble matter.

Some grms. of the sample were extracted with 500 times of its weight of hot water, and the definite volume of this extract was dried up and weighed. The soluble matter also increases according as the course of the pupa stage. This increase means the formation of the substances of lower molecular weight such as amino acids, bases, proteins and uric acid. Although the increase of the uric acid was not determined in this experiment, one can assume that there was increase of the uric acid.

|                    | 1     | 2     | 3     | 4     | 5     | 6     | 7     |
|--------------------|-------|-------|-------|-------|-------|-------|-------|
| % in fresh matter. | 3.73  | 4.28  | 6.38  | 6.39  | 6.75  | 7.38  | 5.89  |
| % in dry matter.   | 15.40 | 18.72 | 28.82 | 28.44 | 30.32 | 34.37 | 19.60 |

### 4. Uric acid nitrogen.

2 grms. sample was extracted with hot water repeatedly and the extract was filled up to 1 liter. From this extract 400 c.c. were taken and the uric acid nitrogen was determined by Krüger and Schmidts' method (Z. physiol. chem. 75. 1. 1905.). From this experiment we found the increase of uric acid nitrogen and uric acid as shown in the next table.

|                     | 1    | 2    | 3    | 4    | 5    | 6    | 7    |
|---------------------|------|------|------|------|------|------|------|
| Uric acid nitrogen. |      |      |      |      |      |      |      |
| % in fresh matter.  | 0.10 | 0.11 | 0.30 | 0.31 | 0.34 | 0.37 | 0.02 |
| % in dry matter.    | 0.42 | 0.48 | 1.37 | 1.37 | 1.57 | 1.72 | 0.08 |
| Uric acid.          |      |      |      |      |      |      |      |
| % in fresh matter.  | 0.30 | 0.33 | 0.90 | 0.93 | 1.02 | 1.17 | 0.06 |
| % in dry matter.    | 1.26 | 1.44 | 4.11 | 4.11 | 4.71 | 5.26 | 0.24 |

From these experiments it is clearly found that the increase of the uric acid at the pupa stage is within the limit of about 1-4 %, and the increase is due to the decomposition of the higher insoluble introgenous matters, and the preparation of the uric acid from the pupa is possible.

(31th. July, 1926)

## ANTINEURITIC PROPERTIES OF ADSUKI BEAN.

By Eiji TAKAHASHI and Kiyoshi SHIRAHAMA.

(Received July 13th., 1926.)

1. Adsuki bean is held in Japan to have a curative effect on beri-beri.

Therefore, the content of the antineuritic vitamine in the bean was studied with its alcohol and water extract upon pigeon, with no marked efficacy. But the normal growth was observed by feeding them with the whole grain of the bean, showing apparently the sufficient content of vitamine B. It is understood that these results must be confirmed with the experimental animals other than pigeon.

2. Allantoin was isolated from the bean and studied of its action upon the polyneuritic pigeon, but no marked effect was observed.

3. The curative potency of the bean on beri-beri will probably be due to another ingredients than vitamine in it. Saponin, found in the bean, may be reckoned as one of them as well.

## ÜBER DIE STICKSTOFFHALTIGEN EXTRAKTIVSTOFFE DER PFERDEHODEN.

Von Kiyohisa YOSHIMURA und Yoshiharu HIWATARI.

(Eingegangen am 2. Aug., 1926.)

Studien über die Bestandteile der Stierhoden sind schon seit langem von vielen Autoren unternommen. L. Leibfreid\* hat aus den Stierhoden Dime-thylguanidin isoliert.

Bei unseren eingehenden Untersuchungen konnten wir in den Pferdehoden Xanthin, Leucin und  $\beta$ -Alanin in kleiner Menge, Cholin und Methylguanidin reichlich isolieren.

1.9 kg. zerhackte Masse der kurz nach der Kastration entnommenen, von der Kapsel befreiten Füllenhoden wurden mit heissem Wasser wiederholt extrahiert, die Auszüge mit Bleiessig gereinigt, dann mit Schwefelwasserstoff entbleit.

Das Filtrat vom Bleisulfid wurde im Vakuum stark eingengt, wobei schied sich 0.3 g. Xanthin aus.

(I) Der Phosphorwolframsäureniederschlag. Die Mutterlauge vom Xanthin wurde mit Schwefelsäure angesäuert und mit Phosphorwolframsäure gefällt.

(a) Die aus dem Phosphorwolframat in gewöhnlicher Weise dargestellte alkalische Flüssigkeit, welche freie Basen enthielt, wurde mit Salpetersäure

\* L. Leibfreid; Zeitschr. f. Physiol. Chem. 139, 82, 1924.

neutralisiert und dann mit Silbernitrat versetzt, wobei ein gelbbrauner Niederschlag entstand; die Menge desselben war aber so gering, dass er zur weiteren Untersuchung nicht ausgereicht hätte.

(b) Der Silbernitrat- und Barythydratniederschlag.

Das Filtrat vom Silbernitratniederschlag wurde mit Silbernitrat und Barythydrat in mässigem Überschusse versetzt.

Der gebildete dunkelbraune Niederschlag wurde mit warmer Salzsäure zersetzt; das Filtrat vom Silberchlorid wurde mit Phosphorwolframsäure gefällt.

Die so gewonnene stark alkalische Flüssigkeit wurde mit überschüssiger Salzsäure angesäuert, eingengt und dann im Vakuumexsikkator stehen gelassen, wobei sich farblose Krystalle ausschieden, die sich nach völligem Austrocknen durch Behandeln mit absolutem Alkohol in folgende zwei Fraktionen trennen liessen:

1. Die in absolutem Alkohol unlösliche Fraktion.

Das Chlorid wurde in das Chloraurat übergeführt; das Chloraurat bestand aus blasgelben Nadelchen mit dem Schmelzpunkt 150–158°C ( $\beta$ -Alaninchloraurat).

2. Die in absolutem Alkohol lösliche Fraktion.

Das aus einem Teil des Chlorides dargestellte Chloraurat bildete gelbe Prismen mit dem Schmelzpunkt 200–204°C (Methylguanidinchloraurat).

Das aus einem Teil des Chlorides dargestellte Pikrat bildete gelbe Nadelchen, die bei 195°C schmolzen (Methylguanidinpikrat).

(c) Das Filtrat vom Silbernitrat- und Barythydratniederschlag.

Das Filtrat vom Silbernitrat- und Barythydratniederschlag wurde in gewöhnlicher Weise wieder mit Phosphorwolframsäure gefällt.

Die aus diesem Niederschlag erhaltene alkalische Flüssigkeit wurde mit überschüssiger Salzsäure angesäuert, eingengt und dann im Vakuumexsikkator krystallisieren gelassen.

Die so erhaltene hygroskopische Krystallmasse wurde in mit alkoholischer Sublimatlösung gefällt. Die Quecksilberdoppelsalze wurden mit Schwefelwasserstoff zersetzt, die gewonnene Chloride im Vakuumexsikkator stehen gelassen, wobei sich farblose hygroskopische Nadeln ausschieden.

Ein Teil des Chlorides wurde in das Chloraurat übergeführt.

Das Chloraurat bestand aus orangegelben Blättchen mit dem Schmelzpunkt 260–254°C (Cholinchloraurat).

(II) Das Filtrat vom Phosphorwolframsäurefällung (I) wurde mittels Barythydrat von Schwefelsäure und Phosphorwolframsäure befreit, der Überschuss des Baryts mittels Schwefelsäure beseitigt und im Vakuum stark

eingedampft, wobei sich kleine Menge von Krystalle ausschieden (Leucin).

Also aus 1.9 kg. frischen Hoden wurden isoliert.

|                                |        |
|--------------------------------|--------|
| Xanthin...                     | 0.3 g. |
| Xanthin-Alanin (Chloraurat)... | 0.3 g. |
| Methylguanidin (Chlorid) ...   | 0.2 g. |
| Cholin (Chloraurat) ...        | 1.5 g. |
| Leucin ...                     | Wenig. |
| NH <sub>4</sub> Cl ...         | 0.8 g. |

## ON THE PHYSICAL PROPERTIES OF "ASAHI PROMOLOID"

By Hideo KANEKO.

(*Chemical Institute, Faculty of Science, Tokyo Imperial University.*)

(Received July 30th., 1929.)

### INTRODUCTION.

The soil, upon which plants take root and thrive, is considered to be a colloid as well as the plants themselves, hence there exist a great common stream of energy between these two worlds.

Recently E. Bottini (Ann. di chim. Appli. **16**, 29 (1926)) made public a very instructive treatise with respect to permeability, capillarity and absorbing power of soils in the presence of Promoloid at the Torino Agricultural Experimental Station in Italy.

He observes that the permeability and capillarity of soils are diminished appropriately in the presence of Promoloid and its absorbing power for ammonium chloride is increased.

I made also a series of experiments on the same subject during the summer in 1924. As the results obtained by me agree with those of Bottini in main respects, I will discuss shortly my results in the following.

Promoloid has good effects upon the growth of many plants in Japan. Especially sugars, starch and phosphoric acid contents in plants are fairly increased by it.

Y. Matsuyama and co-workers (Report of Research Laboratory, Asahi

Glass Co., Ltd., 11, 13, 15, in 1925) showed that Promoloid has a substituting action for phosphoric acid. It is known that colloidal silica, one of the components of Promoloid, displays many important photochemical and biochemical actions; the inversion of cane sugar (Albert et A. Mary, *Compt. rend.* **167**, 644-1918; R. T. Dufford S. Calvert and D. Nightingale, *J. Am. Chem. Soc.* **45**, 2058-1923), the substitute action for  $P_2O_5$  in plants (D. R. Nanji and W. S. Shaw, *J. Soc. Chem. India*, **44**, 1-1925), Selective positive adsorption of  $PO_4$  ion (R. C. Wiley and N. E. Gordon, *Soil Scie.* **14**, 441-1922), positive action for photochemical decomposition of silver bromide (R. Schwarz und H. Stock, *Zeit. Anorg. and Allg. Chem.*, **129**, 41-1923) etc.

The other component magnesium or magnesium oxide also displays many special actions. The former is contained in chlorophyll as an essential constituent which plays a great rôle in the assimilation and seems to synthesise vitamine A from inorganic compounds, (K. H. Coward and J. C. Drummond, *Biochem. J.* **15**, 530-1921.)

In practice, green color of leaves of plant is deepened by Promoloid and 80 % acetone extract of such leaves maintains its green color for a long time.

Magnesium combines with nitrogen at high temperature, but I consider that, under a special catalytic action of ferments or colloidal substances, it may have some favorable relations with nitrogen containing compounds such as protoplasmic protein even at room temperature.

The magnesium oxide has a favorable action for the absorption of phosphoric acid under colloidal state, disperses the soil colloids in smaller particles and makes nitrification more vigorous in the acidic soil.

As physical properties of soil I took into the consideration;—the dispersion of soil particles, absorbing capacity of water, absorbing power of fertilizer and permeability of water. The effect of Promoloid was observed for these factors.

#### EXPERIMENTAL PART.

##### [I] *Field soils:*

300 grams of field soil in natural state as far as possible were put in a large glass funnel which was stopped at its bottom by absorbent cotton. It was moistened properly with rain water and left standing for a day. On the next day, a definite volume (100 c.c.) of rain water or solution containing fertilizer were poured upon the soil. The volume of water passing through it was measured in a cylinder at definite intervals.

## (a) Dry sandy field soil near the Tamagawa, Tokyofu.

Dry state, very coarse grain ;

|  | $V_1$ (c.c.) | $V_2$ (c.c.) | $V_3$ (c.c.) |
|--|--------------|--------------|--------------|
| ( I ) Rain water (100 c.c.)  | 19           | 37.5         | 38           |
| ( II ) Water + Promoloid (0.5 c.c.)                                | 17           | 33.0         | 37.5         |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$                 | 19           | 35.5         | 36           |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid      | 12           | 28.0         | 38           |
| ( V ) Water + 2 gr. $\text{KNO}_3$ + Promoloid                     | 14.5         | 31.5         | 31.5         |
| ( VI ) Water + 2 gr. $\text{Na}_2\text{HPO}_4$ + Promoloid         | 15           | 34.0         | 34.5         |
| $V_1$ ..... Volume of water passing through soil after 10 minutes. |              |              |              |
| $V_2$ ..... " " " " 20 minutes.                                    |              |              |              |
| $V_3$ ..... " " " " 30 minutes.                                    |              |              |              |

## (b) Field soil at Mito :

Wet state, very coarse grain ;

|   | V c.c. (after 5 minutes) |
|---|--------------------------|
| ( I ) Rain water  | 92                       |
| ( II ) Water + Promoloid (0.5 c.c.)                           | 90                       |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$            | 92                       |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 99                       |
| ( V ) Water + 2 gr. $\text{KNO}_3$ + Promoloid                | 97                       |
| ( VI ) Water + 3 gr. $\text{Na}_2\text{HPO}_4$ + Promoloid    | 13                       |

## (c) Field soil at Yoyogi, Tokyofu :

dry state, coarse grain ;

|  | V c.c. (after 30 min.) |
|--|------------------------|
| ( I ) Rain water   | 80                     |
| ( II ) Water + Promoloid (2 c.c. ten times diluted solution) | 73                     |
| ( III ) Water + Oil cake (2 gr.)                             | 61                     |
| ( IV ) Water + 2 gr. Oil cake + Promoloid                    | 74.0                   |
| ( V ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$             | 66.0                   |
| ( VI ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_2$ Promoloid  | 74.5                   |
| ( VII ) Water + 2 gr. Calcium superphosphate                 | 64.0                   |
| ( VIII ) Water + 2 gr. Ca-superphosphate + Promoloid         | 73.0                   |

## (d) Field soil, at Kumagai, Saitama :

Wet state, small grain, compact condition ;

|  | V c.c. (after an hour) | V c.c. (after 2 hours) |
|--|------------------------|------------------------|
| ( I ) Rain water   | 9.0                    | 17.0                   |
| ( II ) Water + Promoloid   | 8.2                    | 16.0                   |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$                       | 23                     | 44                     |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid (0.5 c.c.) | 25                     | 48.5                   |
| ( V ) Water + 2 gr. $\text{Na}_2\text{HPO}_4$ + Promoloid                | 8.5                    | 16.5                   |

## (e) Field soil at Toyonaka, Osakafu :

Wet state, small grain, compact condition ;

|   | V c.c. (after<br>1 hour) | V c.c. (after<br>2 hours) |
|---|--------------------------|---------------------------|
| ( I ) Rain water  | 22                       | 34.0                      |
| ( II ) Water + Promoloid (0.5 c.c.)                           | 20.1                     | 32.0                      |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$            | 26                       | 37.5                      |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 41                       | 42.9                      |
| ( V ) Water + 2 gr. $\text{Na}_2\text{HPO}_4$ + Promoloid     | 20                       | 32.0                      |

( f ) Sandy soil at Tamagawa, Tokyofu :

Wet state, coarse grain, compact condition :

|   | $V_1$ | $V_2$ | $V_3$ |
|---|-------|-------|-------|
| ( I ) Rain water  | 30.5  | 58.6  | 84.5  |
| ( II ) Water + Promoloid (0.5 c.c.)                           | 31.5  | 59.5  | 85.0  |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$            | 33    | 61    | 93    |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 17    | 32    | 46    |
| ( V ) Water + 2 gr. $\text{KNO}_3$ + Promoloid.               | 30    | 53.5  | 73.5  |
| ( VI ) Water + 2 gr. $\text{Na}_2\text{HPO}_4$ + Promoloid    | 26    | 47.5  | 66.5  |
| $V_1$ .....after 10 minutes.                                  |       |       |       |
| $V_2$ .....after 20 "   |       |       |       |
| $V_3$ .....after 30 "   |       |       |       |

[II] *Muddy field soils or Rice field Soils :*

300 grs. of soil, salts and 100 c.c. water were mixed well in a beaker, frequently stirred to a muddy form and left standing for 3 days. The mixture was then poured into a large glass funnel described above and its permeability of water was measured as before.

( a ) Rice field soil, Yoyogi, Tokyofu :

Muddy state, small grain ;

|   | V c.c. (after a day) |
|---|----------------------|
| ( I ) Rain water  | 93.5                 |
| ( II ) Water + Promoloid (2 c.c. ten times diluted solution)  | 96.0                 |
| ( III ) Water + Oil cake (2 gr.)                              | 98                   |
| ( IV ) Water + 2 gr. Oil cake + Promoloid                     | 94.5                 |
| ( V ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$              | 97.5                 |
| ( VI ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 95                   |
| ( VII ) Water + 2 gr. Calcium Superphosphate                  | 98                   |
| ( VIII ) Water + 2 gr. Ca-Superphosphate + Promoloid.         | 95                   |

( b ) Muddy rice field soil, Kumagai, Saitama :

Muddy state, small grain :

|   | V c.c. (after a day) |
|---|----------------------|
| ( I ) Rain water  | 95                   |
| ( II ) Water + Promoloid (0.5 c.c.)                           | 96                   |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$            | 93.5                 |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 83.5                 |
| ( V ) Water + 2 gr. $\text{Na}_2\text{HPO}_4$ + Promoloid     | 92.5                 |

## (c) Muddy rice field soil, Nakasu, Nagano :

Muddy form, very small grain, compact condition.

|   | V c.c. (after<br>1 day) | V c.c. (after<br>3 days) |
|---|-------------------------|--------------------------|
| ( I ) Rain water  | 5.0                     | 30                       |
| ( II ) Water + Promoloid (0.2 c.c.)                           | 5.3                     | 32                       |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$            | 5.5                     | 33                       |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 3.5                     | 37                       |

## (d) Muddy field soil, Hayama, Kanagawa :

Muddy form, very small grain, compact condition.

|   | V c.c. (after a day) |
|---|----------------------|
| ( I ) Rain water  | 9.0                  |
| ( II ) Water Promoloid (0.5 c.c.)                             | 9.5                  |
| ( III ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$            | 23.0                 |
| ( IV ) Water + 2 gr. $(\text{NH}_4)_2\text{SO}_4$ + Promoloid | 15.5                 |

From the above results we obtain the following table.

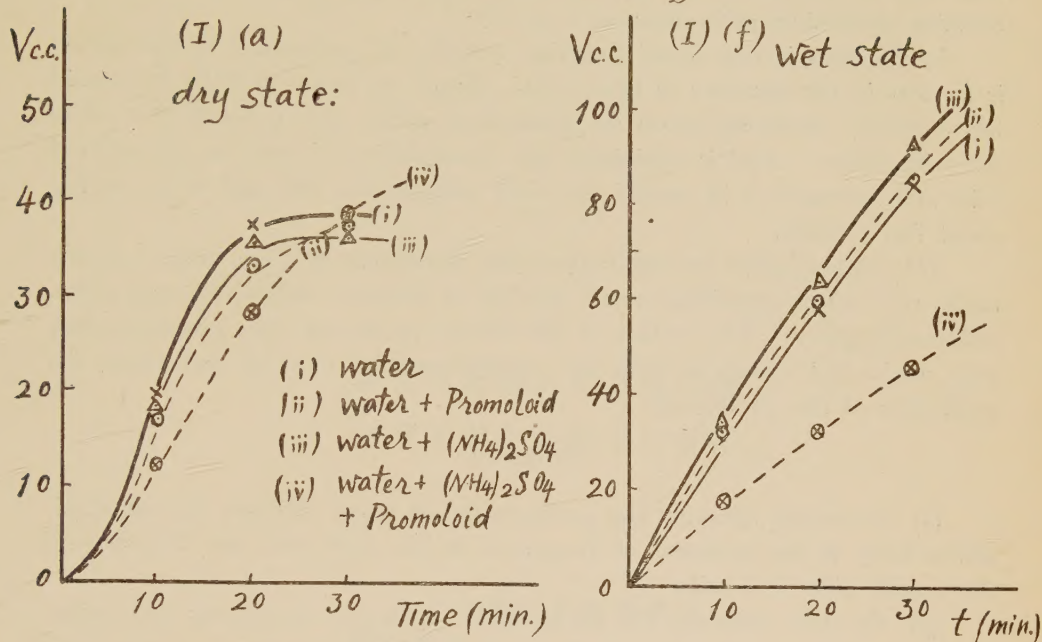
 $V_o$  ..... Volume of water passing through the soil after t minutes. $V_o'$  ..... Volume of water containing Promoloid after t minutes. $V$  ..... Volume of water containing ammonium sulfate after the time t. $V'$  ..... Volume of water containing ammonium sulfate and Promoloid after the time t.

|            | $V_o$ | $V_o'$ | $\frac{V_o'}{V_o}$ | $V$  | $V'$ | $\frac{V'}{V}$ |
|------------|-------|--------|--------------------|------|------|----------------|
| ( I ) (a)  | 19    | 17     | 0.90               | 19   | 12   | 0.63           |
|            | 37.5  | 33     | 0.88               | 35.5 | 28   | 0.79           |
|            | 38    | 37.5   | 0.98               | 36   | 38   | 1.11           |
| ( b )      | 92    | 90     | 0.62               | 92   | 99   | 1.07           |
| ( c )      | 80    | 73     | 0.91               | 66   | 74.5 | 1.12           |
| ( d )      | 9     | 8.2    | 0.91               | 23   | 25   | 1.09           |
| ( e )      | 22    | 20.1   | 0.91               | 36   | 41   | 1.13           |
|            | 34    | 32     | 0.94               | 37.5 | 42.9 | 1.12           |
| ( f )      | 30.5  | 31.5   | 1.03*              | 33   | 17   | 0.51*          |
|            | 58.5  | 59.5   | 1.01*              | 64   | 32   | 0.50*          |
|            | 84.5  | 85.0   | 1.00*              | 93   | 46   | 0.50*          |
| ( II ) (a) | 93.5  | 96     | 1.02*              | 97.5 | 95   | 0.97*          |
| ( b )      | 95    | 96     | 1.01*              | 96.5 | 83.5 | 0.85*          |
| ( c )      | 5     | 5.3    | 1.06*              | 5.5  | 3.5  | 0.63*          |
|            | 30    | 32     | 1.06*              | 33   | 37   | 1.12           |
| ( d )      | 9     | 9.5    | 1.05*              | 23   | 15.5 | 0.67*          |

In all field soils except wet sandy soil (f) the permeability of water through the soil diminishes in the Presence of promoloid. This agrees with Bottini's result. But, the permeability of water which contains ammonium sulfate increases on the contrary in the presence of Promoloid; the increment of permeability being about 10 percent. But oppositely, in wet sandy field

Fig. 1

## Sandy field soils, Tamagawa:



## Muddy rice field soil, Kumagaya

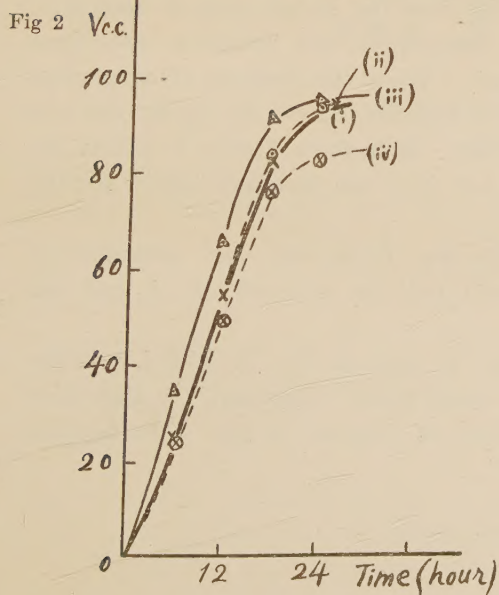
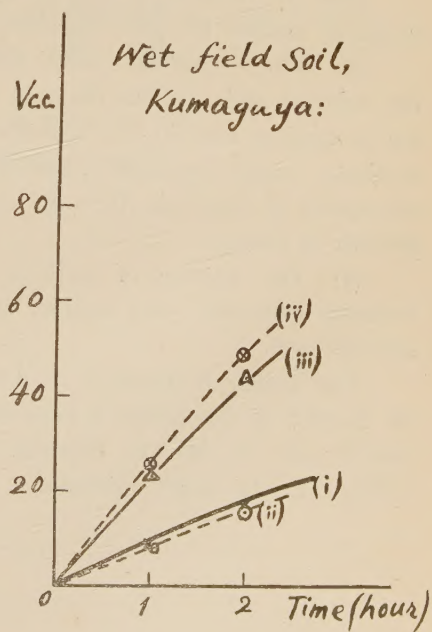


Fig. 3

## Wet field Soil, Kumagaya:



soils and muddy rice field soils the permeability of water increases after a long time in the presence of promoloid and the permeability of water which contains ammonium salts diminishes greatly.

In all cases it was found that the quantity of ammonium in the filtrate is far less in the presence of Promoloid. Hence we know that the Promoloid has a strong absorbing power for ammonium sulfate and prevents it to flow out with water. And it diminishes the Permeability of water in coarse field soils and increases it in muddy rice field soils which fill with water during about four months.

This makes active for the renewal the circulation of water and to absorb easily rain water containing small quantity of nitrogen compounds and radioactive substances. The surface of soil where promoloid was given becomes very rough and seems to help the development of roots by facilitating the ventilation of the air through it.

#### SUMMARY :

(1) Generally speaking the permeability of water through the soil diminishes fairly in the presence of Promoloid in the field soils and increases in the muddy rice field soils.

(2) On the contrary the permeability of water containing ammonium sulfate increases in the presence of Promoloid in the former case and diminishes in the latter case.

This is because it easily absorbs rain, fertilizers and will keep out probably against the decomposition of salt near the surface and its outflow.

(3) The Promoloid easily absorbs ammonium and potassium salts from the solution and reserves them in the soil. When the contents of ammonium and potassium ions in the filtrates were compared, they were by far the less in those come from the soils given with Promoloid. Hence it assists the absorption of manures through roots and give the favorable effects for the growth of plants.

(4) The surface of soils to which the Promoloid was administered, becomes generally very coarse, so it will help the entrance of air and rain into the soil.

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